

Ultrastructural Study of Endogenous Energy Substrates in Spermatozoa of the Sea Urchins *Arbacia lixula* and *Paracentrotus lividus*

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ABSTRACT—Spermatozoa of the sea urchins, *Arbacia lixula* and *Paracentrotus lividus*, use endogenous triglyceride (TG) and phosphatidylcholine (PC), respectively, to produce energy for swimming. The present study examined ultrastructurally the location of TG and PC available for utilization in energy metabolism in spermatozoa of these species. The *A. lixula* spermatozoon contained several lipid globules in the midpiece proximally from the head. After incubation with seawater, TG levels decreased and morphological changes in the lipid globules were observed. Some lipid globules lost their smooth spherical shape, and their surface became irregular and uneven. Vacuoles of various sizes and forms also appeared near the lipid globules. In contrast, *P. lividus* spermatozoa possessed lipid bodies, instead of lipid globules, in the space between the mitochondrial outer and inner membranes. After incubation with seawater, the lipid bodies became small and finally disappeared, coincident with a decrease in the level of PC. These results strongly suggest that TG and PC, as endogenous substrates providing energy for motility, are stored in the lipid globules of *A. lixula* spermatozoa and the lipid bodies of *P. lividus* spermatozoa, respectively.

INTRODUCTION

Sea urchin spermatozoa obtain energy for flagellar movement from oxidation of an endogenous substrate [14–16]. In our recent study, following incubation in seawater, a decrease in the level of endogenous triglyceride (TG) was observed in the spermatozoa of the sea urchin *Arbacia lixula*, which belongs to the order Arbacioida, whereas in spermatozoa of *Paracentrotus lividus*, of the order Echinoida, the content of phospholipids, particularly phosphatidylcholine (PC), decreased [10]. Whereas the spermatozoa of *P. lividus* are generally composed of various phospholipids and cholesterol, *A. lixula* spermatozoa additionally contain TG [10]. The preferential hydrolysis of TG and PC is related to the properties of lipase and phospholipase A₂, respectively [10]. Glycogen and glucose are present in trace amounts in both species [10]. These findings suggest that *A. lixula* spermatozoa obtain energy for swimming through oxidation of endogenous TG, whereas *P. lividus* spermatozoa use mainly PC as a source for energy metabolism. Similar findings have been obtained for spermatozoa of other species of sea urchins belonging to the orders Arbacioida [6] and Echinoida [9, 11, 12]. The energy-metabolic system in sea urchin spermatozoa appears to differ between the Arbacioida and Echinoida.

It has been demonstrated that the sperm midpiece of *Glyptocidaris crenularis* (Arbacioida) contains a single mitochondrion and lipid globules [8]. The lipid globule is

spherical and located in the posterior region between the basis of the mitochondrion and the plasma membrane. Similar lipid globules have been observed in spermatozoa of *Arbacia punctulata* [2, 4] and *Brissopsis lyrifera* [1]. Since there is a concomitant decrease in the level of intracellular TG in *G. crenularis* spermatozoa [8], it is assumed that the lipid globules contain TG, which is available for utilization in energy metabolism.

In contrast, it has been shown that the sperm midpiece of *Hemicentrotus pulcherrimus* (Echinoida) contains several lipid bodies within the mitochondrion [7]. This lipid body differs from the lipid globules, because the former is located inside the mitochondrion and it is relatively small in comparison with lipid globules. Following the initiation of swimming, the lipid bodies in *H. pulcherrimus* spermatozoa become small, coincident with a decrease in the level of PC [7]. Presumably, the lipid bodies within the mitochondria of spermatozoa are reservoirs of endogenous PC substrate. To obtain additional information on the energy metabolism of spermatozoa of the sea urchins *A. lixula* and *P. lividus*, the present study was undertaken to determine whether the sperm midpiece in both species contains lipid bodies or lipid globules. Furthermore, the relationship between these lipid inclusions and energy metabolism was examined.

MATERIALS AND METHODS

Materials

Spawning of stored spermatozoa of the sea urchins *A. lixula* and *P. lividus* was induced by injecting 0.5 M KCl into the coelomic

cavity. Semen was always collected freshly as 'dry sperm' and kept undiluted on ice. The number of spermatozoa was calculated on the basis of protein concentration, which was determined using a Micro BCA protein assay kit (Pierce, IL). The protein content per 10^9 spermatozoa was 0.5 ± 0.1 mg in both species.

Incubation of spermatozoa

Dry sperm were diluted 100-fold in artificial seawater (ASW) consisting of 458 mM NaCl, 9.6 mM KCl, 10 mM CaCl_2 , 49 mM MgSO_4 , and 10 mM Tris-HCl at pH 8.2. After dilution and incubation at 20°C , the sperm suspension was centrifuged at $3,000 \times g$ for 5 min at 0°C .

Determination of PC and TG concentrations

Total lipids were extracted from spermatozoa using the method of Bligh and Dyer (1959). PC and TG levels were determined by high-performance thin-layer chromatography (HPTLC), as described previously [5, 11, 12].

Preparation for electron microscopy

Dry sperm were diluted 100-fold in ASW and incubated at 20°C . At appropriate intervals, the spermatozoa were prefixed in 2.5% glutaraldehyde ASW solution for 40–60 min; a volume of sperm suspension was mixed with the same volume of 5% glutaraldehyde in 80% ASW. The prefixed spermatozoa were rinsed with ASW and post-fixed with 1% OsO_4 for 2 hr. After dehydration in a graded series of ethanol solutions, the specimens were embedded in epoxy resin, and ultrathin sections were cut on a Reichert Ultracut ultramicrotome. After staining the specimens with lead citrate, they were observed using a Hitachi 7000 electron microscope.

Reagents

The TG and PC standards were purchased from Sigma Chemical Co. (St. Louis, MO). All reagents and solvents were of analytical grade. HPTLC plates (silica gel 60) were obtained from E. Merck (Darmstadt, Germany).

RESULTS

It has been demonstrated that the level of TG in *A. lixula* and of PC in *P. lividus* spermatozoa decreases, respectively, after 1 hr of incubation in seawater [10]. Confirming this, the PC level decreased gradually when dry sperm of *P. lividus* had been diluted and incubated for 15, 30, 45, and 60 min in ASW (Fig. 1a). About $23 \mu\text{g}$ PC was contained in 10^9 spermatozoa. During incubation for 1 hr, about $5 \mu\text{g}$ PC was consumed by the spermatozoa. Although *A. lixula* spermatozoa contained PC (about $22 \mu\text{g}/10^9$ sperm), the PC content did not change significantly during incubation (Fig. 1a). In contrast, the level of TG in *A. lixula* spermatozoa decreased following incubation in ASW (Fig. 1b). About $3 \mu\text{g}$ of TG was consumed in 10^9 spermatozoa during incubation for 1 hr. TG was present in a trace amount ($<1 \mu\text{g}/10^9$ sperm) in *P. lividus* spermatozoa [10].

The sea urchin spermatozoon consists of a head, a midpiece and a tail. In longitudinal sections through spermatozoa of *A. lixula*, the midpiece was observed to consist of a single mitochondrion and several lipid globules (Fig. 2a). All spermatozoa contained these lipid globules, which were

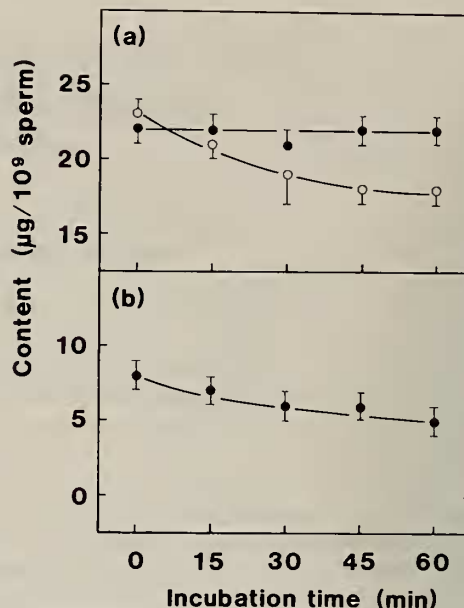


FIG. 1. Changes in levels of phosphatidylcholine (a) and triglyceride (b) in *A. lixula* (●) and *P. lividus* (○) spermatozoa following incubation in seawater. Dry sperm were diluted 100-fold and incubated in seawater at 20°C . Each value is the mean of four separate experiments. Vertical bars show S.E.M.

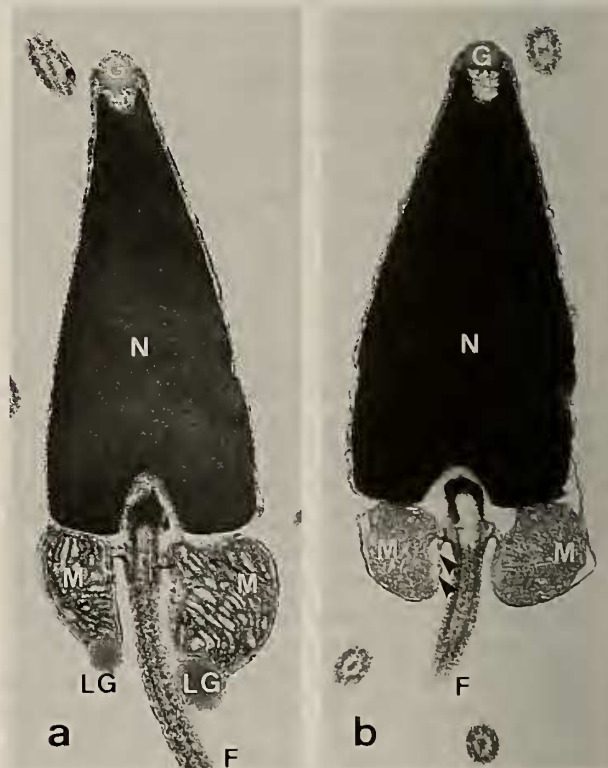


FIG. 2. Longitudinal section through a spermatozoon of *A. lixula* (a) and *P. lividus* (b). Arrow heads show lipid bodies. F: flagellum, G: acrosomal granule, LG: lipid globule, M: mitochondrion, N: nucleus. $\times 25,000$.

mostly spherical and homogeneously dense in appearance, and located distally in the sperm midpiece (Fig. 3a), distributed in a band nearest the flagellum (Fig. 3b).

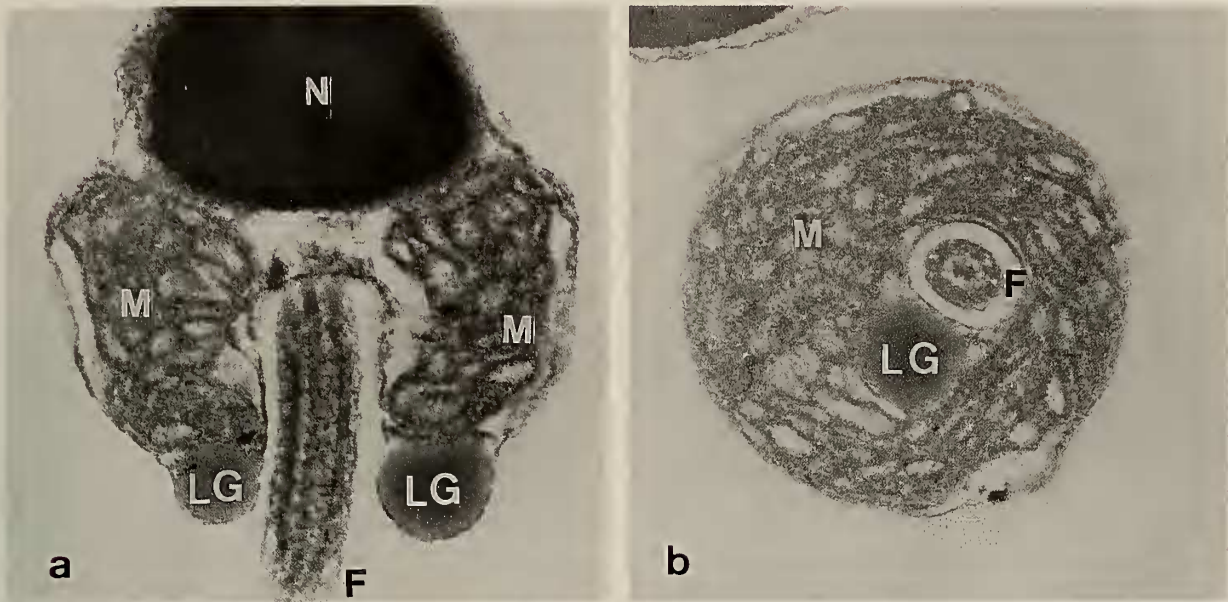


FIG. 3. Longitudinal (a) and transverse (b) sections through the mitochondrial region of *A. lixula* spermatozoa before incubation in seawater. F: flagellum, LG: lipid globule, M: mitochondrion, N: nucleus. $\times 55,000$.

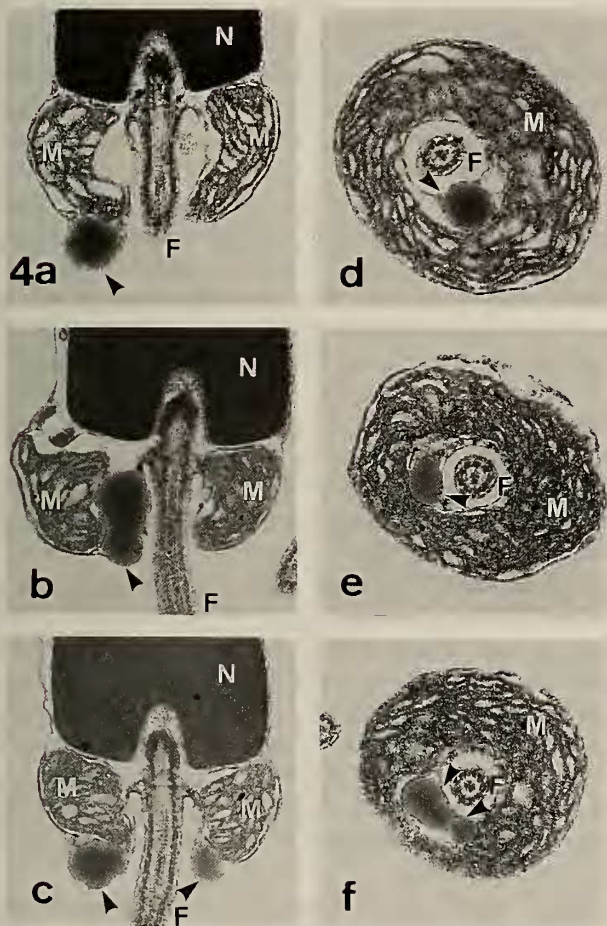


FIG. 4. Longitudinal (a-c) and transverse (d-f) sections through the mitochondrial region of *A. lixula* spermatozoa after incubation in seawater for 10 min (a, d), 30 min (b, e) and 60 min (c, f). Arrow heads show lipid globules. F: flagellum, M: mitochondrion, N: nucleus. $\times 27,000$.

Longitudinal and transverse sections of the midpieces of *A. lixula* spermatozoa were examined after incubation in ASW. After incubation for 10 min, there was little evident change in the lipid globules (Figs. 4a and d). After 30 min of incubation, some lipid globules had lost their smooth spherical shape, and their surface had become irregular and uneven (Figs. 4b and e). After 60 min of incubation, irregular and uneven globules appeared frequently (Figs. 4c and f).

In contrast to *A. lixula*, the midpiece of the *P. lividus* spermatozoon did not contain lipid globules (Fig. 2b). A region between the mitochondrial outer and inner membranes was dilated in a band nearest the flagellum and contained low-electron-density lipid bodies (Fig. 5), similar to those observed in the spermatozoa of *H. pulcherrimus* [7]. These lipid bodies were irregular in profile and smaller than lipid globules. After 10 min of incubation in ASW, lipid bodies were still present (Figs. 6a and d), and a gap was also observed to have opened between the plasma membrane and the mitochondrial outer membrane. At 30 min after incubation, the lipid bodies had become small (Figs. 6b and e) and by 60 min, the lipid bodies and inner ring of the mitochondrion had finally disappeared (Figs. 6c and f). However, various structural features of the mitochondrion, such as the number of cristae and the thickness of the membranes, did not change during incubation in ASW.

DISCUSSION

This study showed that lipid globules were present in spermatozoa of *A. lixula* of the order Arbacioida (Fig. 2a) and that lipid bodies were present in those of *P. lividus* of the order Echinoida (Fig. 2b). The lipid globules were located distant from the mitochondrion, in the midpiece proximally from the head (Fig. 3), whereas the lipid bodies were distri-

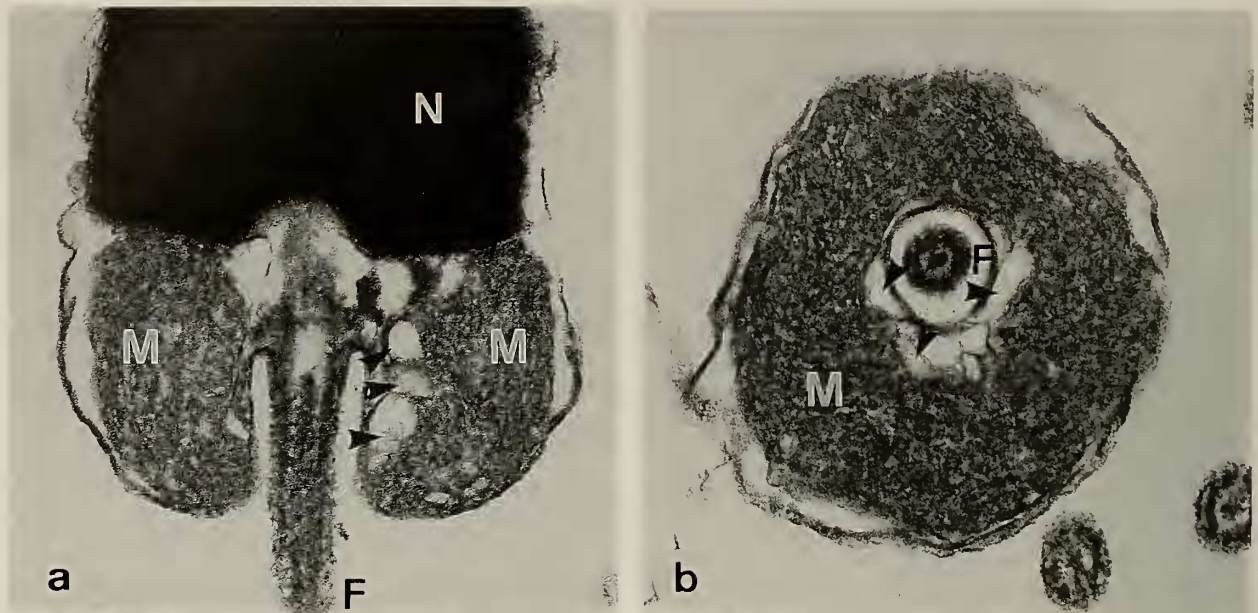


FIG. 5. Longitudinal (a) and transverse (b) sections through the mitochondrial region of *P. lividus* spermatozoa before incubation in seawater. Arrow heads show lipid bodies. F: flagellum, M: mitochondrion, N: nucleus. $\times 55,000$.

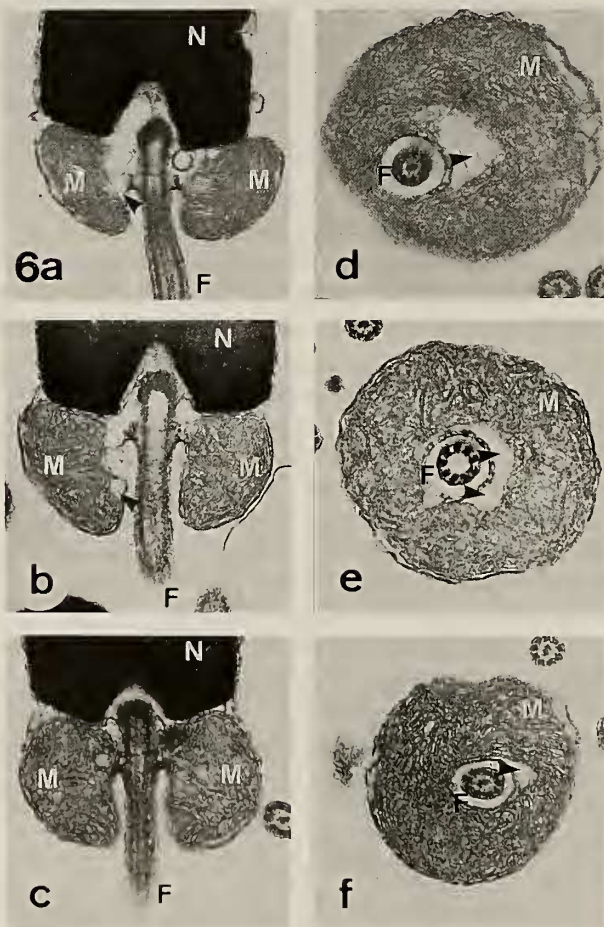


FIG. 6. Longitudinal (a-c) and transverse (d-f) sections through the mitochondrial region of *P. lividus* spermatozoa after incubation in seawater for 10 min (a, d), 30 min (b, e) and 60 min (c, f). Arrow heads show lipid bodies. F: flagellum, M: mitochondrion, N: nucleus. $\times 27,000$.

buted within the mitochondrion (Fig. 5). Morphological changes in both the lipid globules (Fig. 4) and lipid bodies (Fig. 6) were observed after incubation, suggesting that digestion of these inclusions occurs simultaneously with activation of energy metabolism.

The several lipid globules were found to be mostly spherical in the midpieces of *A. lixula* spermatozoa before incubation (Figs. 3 and 4), but they became irregular and uneven after incubation. Previous studies have shown that the total volume of lipid globules in spermatozoa of *G. crenularis* is reduced during incubation, concomitantly with a decrease in the level of TG [8]. The present study also revealed a decrease of TG levels in spermatozoa of *A. lixula* after incubation (Fig. 1b), as described previously [10]. Therefore it is possible that TG available for utilization in energy metabolism is contained in the lipid globules of *A. lixula* spermatozoa.

In contrast, PC is the substrate used for energy metabolism in *P. lividus* spermatozoa [10]. This study also showed that the lipid bodies shrank and disappeared (Fig. 6) in parallel with changes in the level of PC (Fig. 1a). These observations suggest that PC available for use in energy metabolism is related to the appearance of lipid bodies. Similar findings have been obtained in spermatozoa of other sea urchins of the order Echinoidea [7, 9]. Thus possibly, the lipid bodies within mitochondria are reservoirs of PC as an endogenous substrate.

With regard to the substrate used for energy metabolism in sea urchin spermatozoa, it is interesting that TG and PC are stored in lipid globules and lipid bodies, respectively. Neither TG nor lipid globules are present in spermatozoa of Echinoidea sea urchins, such as *P. lividus* (Figs. 1 and 2) and *H. pulcherrimus* [7]. Therefore it is assumed that the Echi-

noida spermatozoa, which have lost their lipid globules, are provided with a system for metabolism of phospholipids, particularly PC, in lipid bodies within mitochondria.

ACKNOWLEDGMENTS

This work was supported by the Japanese Society for Promotion of Science (JSPS) and the Italian National Research Council (CNR).

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